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ANTHRAX STUDIES

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(From the Hygienic Institute of the German University in Prague; Professor O. Bail, Director.)

Study prepared with the support of the Ministry of Agriculture.

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Bacteriologists have always been most interested in anthrax infections because there is probably no other infectious disease where the situation is as complicated in every respect as in this particular one. The genesis of the disease itself and the death of the usual experimental animals due to infection create difficulties in finding an explanation and the entire problem of septic disease is one of the most difficult in the field of infectious diseases altogether. In most other infections, sickness and death can be explained on the basis of the toxins produced by the particular bacillus, regardless of whether these are genuine toxins or whether they are toxic body substances; in contrast to this, no toxins of any kind have ever been found in the group of the aggressin sources, which also includes the anthrax bacillus and the most virulent microorganisms; in other words, no toxins were ever found to which the disease could have been traced with any degree of certainty. There have been many attempts to establish the presence of anthrax toxin but these projects never came up with any reliable results; this also applies to the work of Hoffa whocstarted with the assumption that the anthrax bacillus first of all releases a toxic decomposition product from the albumin substances in the infected animal; he assumed that this toxic decomposition product produces the disease. Hoffa was able to isolate an alkaloid-like toxin from cultures of anthrax bacilli on meat; but this toxin did not differ from the albumin decomposition products which result from different bacterial decomposition and could therefore not be used in explaining the specific anthrax disease as such.

The situation was roughly the same in the case of the physical explanation for this disease; here we might mention primarily two: one of them believes that the withdrawal of oxygen, which the organism suffers as a result of the microbes proliferating inside it, is the cause of the disease; the other theory emphasizes the inhibition of circulation by the bacilli which clog the capillaries. Although both of these theories, even when combined, cannot satisfy us fully, they nevertheless enable us to recognize an important change in the concept of the infectious disease. What we have here for the first time is an attempt to explain an infectious disease without trying to blame it on any specific toxins. In other words, both theories point to the assumption that the bacilli -- without in any way interfering in the regimen of the organism through metabolism products harmful to it, inflict damage upon the organism by virtue of their mere presence, in that they inhibit certain functions.

The question as to immunity and natural resistance was just as perplexing until Bail's fundamental discoveries -- involving the location of

the aggressins and the antiaggressins -- threw some light on hitherto unanswered questions. The fact that the anthrax bacillus gives off substances in the animal body which, without producing intoxication symptoms, overcome the anthracocidal forces of the living organism, until it is helplessly exposed to the invasion of the bacilli, the fact that the bacillus, which is present in the body of the immune animal, containing antiaggressins in its serum, is deprived of these attack substances and is turned into a saprophyte, which easily succumbs to the bactericidal properties of normal animal tissue -- this fact for the first time offered a satisfactory explanation for immunity.

The question as to the natural resistance of certain animal species led to some very vehement debates, between the German and the French school. The question as to whether the cell effect, that is to say, phagocytosis, or the juice bacteria-killing process, that is to say, alexin, was discussed at length by Metschnikoff, on one side of the fence, and Buchner on the other side of the fence; but this debate did not lead to any clear result. It turned out that the anthrax-killing capability [or process] of the organism -- regardless of whether we are talking about the plakins, leucocytes, or the juice bacteria-killing process -- could not in any way be tied in with the resistance or receptivity of the particular animal species. Here again, it was the work of Bail and his school which finally explained the rather contradictary findings. Bail first of all with the help of test tube experiments had traced the natural resistance of the dog to the fact that an antibody-like substance is present in the dog serum and that this substance, completed by leucocytes, develops anthrax-killing properties and that, moreover, the leucocytes, which, according to the investigations of Gruber and Futakis, cannot phagocytize encapsulated animal bacilli, but can kill animal bacilli through soluble substances, through the aphagocidal process studied in great detail afterward by Weil; after that, Bail and Weil and Suzuki also explained the resistance. These investigators were able to prove that leucocyte bacteria-killing processes against animal bacilli -- for instance, guinea pig leucocytes -- are in no way weaker than the process developed by leucocytes of resistant animal species, such as pigeons or dogs, through which the aggressins, given off by the antrax bacilli, are quickly rendered ineffective; what happens here is that the aggressins paralyze the anthrax-killing agents or that the exit of these anthrax-killing agents from the cells is prevented, while the leucocytes of naturally resistant animal species can develop their germ-killing properties also when aggresin is added. They were also able to prove that the difference between the resistant and the receptive organism, for instance, the pigeon and the guinea pig, is to be found not in a bacteria-killing process -- developed in the pigeon organism -- which is stronger from the very beginning but rather in the preservation of the bacteria-killing capability in the bird cell; on the other hand, the guinea pig organism, whose bactericidal forces were rendered ineffective through aggressin in a very short time, is helplessly exposed to the bacilli after this time.

These investigation results contain two extremely important findings. It was proved here for the first time that immunity and resistance against

anthrax are identical as to their essence. The basic difference here is not whether the anthrax-killing capability [process] of the organism is assured by the fact that the cells of the resistant animal keep their germ-killing properties, even when the bacillus secretes aggressins, or whether the aggressins in the receptive but immunized animal are again and again neutralized by the antiaggressins and whether this preserves the germ-killing properties of the cell. The most essential aspect in both of these forms of reactions is the preservation of the anthrax-killing capability in the cell which in one case produces the immunity and in the other case brings about the resistance observed.

Furthermore, this investigation result also contains an indirect, implied but extremely important finding with respect to the pathogenesis of anthrax in the broader sense of the septic infectious disease as such; what we have here is the finding that an in itself harmless bacteria product, such as aggressin, nevertheless can inflict irreparable damage to the body through the specific paralyzation of the aphagocidal force of the cells -- without there being any possibility of speaking of a toxic effect as such. This furthermore shows how a circle is formed between the bacillus and the aggressin -- a circle which in the end is bound to lead to the death of the animal. The aggressin, at first produced in a very small quantity, creates the condition for the multiplication of the bacteria. The bacilli then secrete larger quantities of their attack substance and this goes on and on until the entire organism has been flooded with aggressin and the bacilli following it. This gives us the idea of the formal genesis of the infectious disease, without their being any need to assume a toxic effect on the part of the bacillus. This leaves only the final stage unexplained -- the sickness stage, in other words, which, in case of experimental anthrax infection, sets in shortly before the animal's death. The duration of the disease quite often is confined to a few minutes or perhaps a quarter of an hour although numerous bacilli are circulating in the blood of the animal hours before that. This behavior speaks against the toxicity of the bacillus -- quite apart from the fact that nobody ever managed to find toxins in the juices or organs of animals suffering from anthrax. The behavior of animals infected with anthrax gives us the impression that the specific paralysis of the anthrax-killing mechanism, described by Bail and Weil, is not confined to this single function of the total organism; instead we get the idea that this paralysis, which progresses as the infection continues, also involves other cell functions which are vital but whose termination or loss can be tolerated for some time until, in the end, the animal simply cannot go on living.

This leads to the question as to which organ is supposed to be hit by this hypothetical damage. The dissection reports on animals struck with anthrax pointed to the parenchymatose organs which were found to have been altered most seriously; but many earlier investigators were never able to find a serious functional impairment of any kind in an animal that had been infected with anthrax but that had not yet come down with the disease. Now, the author and H. Adler, studying pneumococci and Bass, studying streptococci, were able to find that the reticulo-endothelial system plays a very important double role in infection with these viruses, that this system is the first

point of attack of the virus in the body, and that it represents the seat of immunity also in the immune animal. They found that the leucocytes do phagocytize in the test tube and in the exsudate but that they do not have this property or that they have it only in a rudimentary fashion in the healthy tissue. The only cells which absorb and kill the viruses in the tissue are the cells of the histiocytary apparatus and consequently the damage due to the infection -- something which the author, by the way, pointed out repeatedly -- is bound to have an effect first of all and even from then on mostly on the reticulo-endothelial system. Following up these reports, it was therefore obvious to examine the function of the reticulo-endothelial system in the incubation of the disease; rapid anthrax, with its long incubation and short disease time, offered an ideal experimental object for this purpose. Of course, it was first necessary to find a method for functional testing [for testing the functions], but Saxal and Donath had already published reports which seemed to indicate that such a method could be found. These two authors reported that animals, blocked with colloidal silver, revealed a delayed [slowed] absorption of stains [dyes] from the blood, in other words, that one could conclude, on the basis of the faster and slower disappearance of colloidal stains injected into the blood, as to what the momentary functional state of the reticulo-endothelium might be. These experiments were checked against animals blocked with India ink and could be confirmed. Such animals revealed a slowed absorption of the Congo red, used in these experiments, from the serum, although during the first period after the injection of the stain, in the case of the blocked animal, the stain content in the serum was found to drop faster than in the normal animal; at any rate, the stain absorption later on was considerably delayed. The more rapid disappearance of the stain during the first minutes after the injection is not very easy to explain; we are probably dealing here with an adsorption of the Congo red into the carbon particles located in the cells of the reticulo-endothelial system; this was pointed out by Haurowitz on the occasion of a debate conducted at the Lotos medical-natural science club. These experiments established that any damage to the reticulo-endothelial system would be indicated by the delayed absorption of the Congo red. We then tested the function of the reticulo-endothelium of animals infected with anthrax in this fashion.

Method: The rabbits are injected with 1 ccm of 1 % Congo red solution, intravenously; after five minutes we bleed the animals and we determine the quantity of Congo red in the serum using the Autenried colorimeter. All of the following values are then related to this first value which is set at 100. The curves therefore indicate merely the resorption speed.

As we can see from the experiments shown here, we find serious damage to this system; this damage can be established even with this certainly very crude method at a moment when there is not the slightest thought of a disease. As little as 16 hours after the infection with a certain quantity of viruses -- which kills the animal after only 4-5 days -- we found a rather increased slow down in the absorption of the Congo red from the blood -- slower, in other words, than the absorption that could have been produced through the most intensive India ink blockage of the reticulo-endothelial system.

Characteristically, we do not observe a rapid drop in the stain quantity during the initial period after the infection in the case of infected animals; this was pointed out during the discussion of the behavior of the blocked animals. The paralysis of the reticulo-endothelial system is very pronounced after as little as 24 hours and keeps increasing as the infection progresses. If we realize that the paralysis of the reticulo-endothelial system -- which, by the way, can readily be recognized from the course of the intravenous infection-- is certainly for the most part specific and we furthermore keep in mind that the absorption of Congo red from the blood cannot be considered as a normal function of the reticulo-endothelial system -- something that was done only because we had no better method for the functional testing of this system -- then we must admit that this damage to the system must be extremely severe already during the very early stages of the infection. This is for the most part analogous to the findings of Bail and Weil and Suzuki where this damage is certainly directed specifically, that is to say, against the anthrax-killing and phagocytary force of this cell system, although this process can also spread to other functions in a very pronounced form. The assumption made initially to the effect that the paralysis of the cells is bound to spread to functions other than their anthrax-killing function can be confirmed on the basis of the results of these experiments for the cells of the reticulo-endothelial system, likewise; we will discuss this again and again in the course of this article in connection with the discussion of the significance of this cellular system for immunity and resistance. This leaves us with the following question to be answered: Can we explain the anthrax disease as such on the basis of this damage to the reticulo-endothelial system? We have a wealth of detailed studies available on the role which this cellular system plays in the management of the organism (Bibliography in Aschoff, in Ergebnissen der inneren Med., Kinderheilk. (Results of Internal Medicine and Pediatrics), 1924); these reports indicate that this particular cell system plays a very important role in intermediate metabolism. It is connected with the blood decomposition and blood formation, with hemoglobin, iron, and lipoid metabolism, with diseases of the liver, and so on. Saxl and Donath even credit it with a role in the water exchange between the blood and the tissues. This all tells us very clearly that damage to this system, as severe as in this case, as the case of this infection, is bound to inflict heavy damage upon the entire metabolism of the infected animal and that it can alter the metabolism of the infected animal in a direction that we might perhaps not be able to define in any greater detail at this time and that it might perhaps even make the metabolism entirely impossible in some other respects. As we said before, this paralysis is certainly for the most part specific; this means that various clinical pictures from different types of damage to the system might also be explained. By the way, the differences in the clinical picture of pure sepsis are not so profound that this kind of assumption would be absolutely necessary. We want to mention all of this at this point only as a purely hypothetical conclusion because, considering the present state of knowledge on the physiology of the reticulo-endothelial system, we cannot make any final statements about the functional failures and phenomena caused by a paralysis of this system. This can be covered only in more detailed studies; the same applies to the more theoretically important question as to whether aggressin or the bacillus

or both of them together produce this paralysis; this question cannot be answered at this time for some external reasons. It might suffice at this point to emphasize the intensive damage to this system during anthrax infection and during septic infection, altogether, which is the first symptom of this type of infection.

The author and H. Adler, in their investigation of pneumococcus immunity, were of the opinion that the reticulo-endothelial system is the main carrier of this immunity, and that serum antibodies, leucocyte bacteria-killing capabilities, etc., at best play a supporting role. The essence of immunity was considered to reside in a specific allergic alteration of the histiocytary cellular system which enables these cells to capture viruses circulating in the blood quickly, to hold them in their interior, and to dissolve them. In a study published on the same topic a short time after our study, Neufeld and Meyer advocate a somewhat different viewpoint. These authors likewise, on the basis of their experiments involving white mice, arrive at the result that the reticulo-endothelial system is the seat of immunity; but when it comes to their concept of the essence of immunity, the views of Neufeld and Meyer do not agree with ours. These authors conclude from the fact that the serum of immune mice, which normally does not contain any antibodies, assumes a high protective value after the injection of of manganese salts, that the immune bodies are preformed in the cell and that they are given off to the blood only in response to the stimulus from the manganese. We can only partly agree with this view. There is certainly a change in the chemistry of the cell as a result of immunization; but our immunization experiments with immune organs and our through-flow experiments prove that we could not possibly be dealing with antibodies formed in the cells here. These experiments -- in which it was impossible to find either a protective value on the part of the immune organs or a stronger filtration of the liquid which contains the cocci and which flows through the immune surviving organ -- in our opinion clearly prove that the essence of the immunity is to be found in an alteration of the living cell which has nothing to do with the cell-grown antibodies, that is to say, with the inanimate reaction products of the cell. In addition we were able to prove in our work on immunity in pneumococcus Type 1 and 2 that the antibodies in the actively immune animal do not get a chance to have any effect at all and that the passive immunity differs from the active immunity through the alteration of the reticulo-endothelium apparatus in one essential point; this alteration is the one that is found in the active immunity. This finding was explained, among others, by the fact that passively immune animals destroy the same cocci quantity in a shorter period of time than the actively immune serum donor. Of course, the experiments involving cocci with their bacteriotropic immunity could not support our view completely and without any doubts. On the other hand, this view would seem to be sufficiently justified and explained if the same behavior can be found in the case of anthrax immunity. In the case of this anthrax immunity -- where there are no bacteriotropic antibodies at all, where the only protective substances that can be proved to exist in the serum of immune animals are directed not against the bacillus but only against its aggressin -- it would seem that a pneumococci infection with a similar infection course in immune animals would necessarily have to be

tied in with an alteration of their reticulo-endothelial system in the sense described earlier -- if we assume that the characteristic features of this immunity are represented by the rapid disappearance of the viruses in connection with intravenous infection, the phagocytosis in the histiocytes which can be found only in the immune animal as a result of bone marrow puncture, the continued limited condition of the viruses in local infection, and finally the weakening of immunity through the blockage of the reticulo-endothelial system. It is quite obvious that antibodies such as the anti-aggressins -- even if they were to develop in the cells -- could bring about neither the rapid disappearance of the viruses injected into the blood stream nor a phagocytosis in the histiocytes and that a blockage would also have to be without any effect because the viruses, deprived of their attack substances through the serum antibodies, would have to be killed even if the reticulo-endothelial system were partly disconnected /eliminated, paralyzed/ which would mean that the defensive forces of the organism, such as leucocyte-killing processes and juice bacteria-killing processes, would have to be slightly different. We will now try to prove that these conditions actually prevail; before we do that we should discuss some of the difficulties which arise when we work with highly-virulent anthrax.

The strain which was used in all of the experiments mentioned in this study turned out to be almost absolutely virulent. We were repeatedly able to find that rabbits weighing 2 kg died of an infection with 20-50 bacilli, accompanied by typical anthrax sepsis. The kind of experiments to be conducted here means that we had to work with large quantities of bacilli -- by the way, bone marrow infections require the injection of large quantities of bacilli; this is why it was difficult to achieve a sufficiently high immunity in the experimental animals. Indeed, despite the great care taken during immunization, a number of animals died of the infection because the infection quantity and the immunity were out of proportion. We performed the immunization in the following fashion: Following the tried and proven directions given by Bail, we first of all produced basic immunity by means of sterile aggressin; this basic immunity was then increased by means of carefully dosed injections using fully virulent bacilli and finally the nonsterilized edema liquid from animals that had died of anthrax. We might explain this with the help of an example. A rabbit is given

First day 10 ccm sterilized aggressin subcutaneously,

10th day 10 ccm sterilized aggressin subcutaneously,

20th day 320 bacilli subcutaneously,

30th day 800 bacilli subcutaneously,

40th day 1,200 bacilli subcutaneously (rather strong edema 3 days after infection),

50th day 1,200 bacilli subcutaneously,

60 th day 2,000 bacilli subcutaneously,

70th day 2 ccm nonsterilized edema liquid with a quantity of bacilli that can no longer be counted, subcutaneously,

80th day 4 ccm.

All of the animals used for these experiments were immunized roughly according to this schedule; these animals also met all of the other requirements as regards immunity. In addition we took care that a period of 2-3 weeks would pass, each time, between the infections performed on the individual animals for experimental purposes. Experiments conducted during shorters intervals of time can cause the death of the experimental animal because bacilli have remained behind in the animal from the last infection. If we work with longer intervals, then we can have no assurance that the laboriously increased immunity will really prevail and continue on this level.

During our discussion of pneumococci immunity we emphasized that the course of the infection in case of intravenous injection reveals a typical difference between normal and immune animals. We can get an idea of the particular immunity state by means of intravenous infection and control of the course of the infection; this would be the best way to get the proper picture of what happens here. This is why we began the investigations on anthrax immunity likewise with intravenous infection experiments.

Experiment 3

An immune and a normal rabbit were administered, each, 0.05 ccm Bouillon culture in 1 ccm cooking salt solution, intravenously. Blood samples were taken from the jugularis. Germ cound in ccm.

	Immune animal	Normal animal
5 Min.	0	160
30 "	0	0
1 hr.	0	0
8 hr.	0	0
24 hr.	0	260
36 hr.	0	∞ (1)
48 hr.	0	
	Continues to live	Dead

This experiment shows us that the tissues, respectively, the reticulo-endothelial system play the significant double role which was found in the previously mentioned investigations as far as pneumococci immunity is concerned; we find that this double role applies also in the case of anthrax infection here. For the case of highly virulent anthrax we can likewise not assume a primary increase in the flowing blood; intead, the reticulo-endothelial system must provide the nutrient medium for this virus likewise.

(1) At this point we must note that a blood plate [slide] overgrown with anthrax colonies, very soon gives the impression as if we had an infinite number of colonies growing here, which could be explained by the discoloration of the blood through the colonies, as well as the size and the rather fuzzy outline of these colonies.

In these experiments, by the way, it is rather interesting to note that the reticulo-endothelium of the normal animal preserves it capability for inhibiting the bacilli for a comparatively long period of time; after that, however, there is a sudden rapid increase which causes the animal to die within a few hours. As for the opinion that the reticulo-endothelium of the normal animal likewise has the capacity for absorbing fully virulent anthrax, one might object that an absorption of culture bacilli, which are so much more liable to be wiped out by phagocytosis, does not prove anything at all. But as we can see from the next experiment, in capsulated animal bacilli are likewise absorbed by the reticulo-endothelium of the normal animal and they get back into the blook only afterward.

Experiment 4

We centrifuge 2 ccm of rabbit. The deposit, which contains a vast number of bacilli and a few red and white blood corpuscles, is then washed twice with a physiological cooking salt solution; then it is mixed with 4 ccm NaCl and half of this amount is injected into one animal, each.

	Immune animal	Normal animal
5 Min.	85	820
30 Min.	0	74
1 hr.	0	10
4 hr.	0	0
30 hr.	0	∞
48 hr.	0	
	Lives	Dead

This experiment shows that the absorption of animal bacilli both in the immune and in the normal animal is considerably slower than the phagocytosis of the culture bacilli; here again, however, we could not determine a primary increase of the bacilli in the flowing blood. The animal bacilli likewise are filtered through the parenchymatose organs of the nonimmunized into the blood several hours later. The death of the animal here again occurs a short time after the penetration of the bacilli into the blood stream.

The second criterion of this immunity -- the fact that the viruses continue to remain limited to the locus of infection in the immune animal whereas they sooner or later occur in the blood of the normal animal -- could likewise be observed in the case of anthrax infection.

Experiment 5

Infection with 0.05 ccm, each, of Bouillon culture, intratracheally.

	Immune animal	Normal animal
1 hr.	⊖	⊖
3 hr.	⊖	⊖
8 hr.	⊖	⊖
24 hr.	⊖	680
36 hr.	⊖	∞
48 hr.	⊖	Dead

The following experiment shows that the peculiarities described for the local infection also apply to encapsulated bacilli.

Article 6

We centrifuge 2 ccm of rabbit edema; the deposit is washed and is then injected in equal parts, subcutaneously.

	Immune animal	Normal animal
2 Hr.	⊖	⊖
4 hr.	⊖	⊖
8 hr.	⊖	⊖
24 hr.	⊖	∞
48 hr.	⊖	Dead

The animal likewise are retained at the locus of the infection surprisingly long and presumably get into the bloodstream only after the defensive forces of the organism have been overcome. Of course, we must emphasize that even before bacilli are found in the flowing blood, we can establish the presence of such bacilli in the parenchymatose organs with the help of cultures. The stage of anthrax sepsis with bacilli in the blood should therefore be considered only as a terminal stage which occurs at a moment when the organism is defensely against the bacilli.

The experimental results described enable us to assume -- parallel to the findings on pneumococci and streptococci -- that the reticulo-endothelial apparatus would also seem to play the main role in anthrax infection. The proof that this assumption is correct had to be furnished here again with the help of bone marrow infection. After several failures caused by the fact that the animal was given either too many bacilli or died of the infection or that we injected too few bacilli and therefore were unable to establish the presence of any bacilli with the microscope, we managed to get the first usable preparations. We found that the anthrax bacilli likewise are phagocytized only in histiocytes.

Experiment 7

Immune animal.

We take a 16-hour diagonal agar culture and we rinse it in 10 ccm NaCl; we distribute it finely and we then inject 0.1 ccm of it into the bone marrow of the tibia.

We remove after 7 hours. Culture positive.

Staining of smears according to Giemsa.

Considering the small infection quantity, we could not really expect anything else and we therefore only found very few anthrax bacilli most of which were already phagocytized in the histiocytes. We find all stages of dissolution, from the still nicely stainable encapsulated bacillus all the way to the hardly recognizable bacillus shadow. We usually have 2-3 rods in a cell and very rarely we get as many as 10-20. All phagocytizing cells appear to be seriously stimulated [irritated], including those which have taken on only one or two bacilli; they reveal collosal nucleus enlargements and to some extent also pyknosis. We were not able to find any phagocytosis in any other cells.

The extracellular bacilli are almost all still well preserved and they have broad, nicely stained capsules and reveal all of the characteristics of the animal bacillus.

The animal continues to live without any serious disease phenomena.

Normal animal.

Same method as above. Culture positive.

In the bone marrow we find a moderate amount of well-capsulated bacill' threads, most of them in a free position. We looked at many dish preparations and were only able to find bacilli twice; these appeared to be partly enclosed in histiocytes. The animal dies every 36 hours after infection; death is due to typical anthrax sepsis. Bone marrow smear after death: vast number of animal bacilli, no phagocytosis.

We were able to pin down the significance of the reticulo-endothelial system likewise for anthrax immunity by means of bone marrow puncture. The anthrax bacilli injected into the bone marrow are absorbed by the histiocytes and are gradually digested in their interior. We were able to recognize phagocytosis also in the non-immune animal, although this was a very minor phagocytosis at that; this is probably due to the fact that the culture bacilli, as we know, very easily succumb to phagocytosis and are turned into the encapsulated animal bacilli only in the interior of the cell. We might also mention that in the immune animal even cells that have absorbed only very few bacilli already appear damaged while, for instance,

in the case of pneumococci phagocytosis we were able to recognize serious damage only when one cell had taken on 10-30 cocci. These findings, which do leave a certain amount of leeway for subjective interpretation, should not be overemphasized, of course; nevertheless, it seems as if the irritation [stimulation] of the reticulo-endothelial system by the anthrax bacilli is much more intensive than when this is brought about by pneumococci or streptococci.

In the experimentation method described, we were able to establish phagocytosis only in the bone marrow histiocytes; a rather accidental finding, however, gave us a general picture of the entire reticulo-endothelial system.

Experiment 8

A normal rabbit was infected subcutaneously with 165 bacilli for other purposes and revealed the following atypical infection course.

1 hr.	0	
5 "	0	
8 "	0	
24 "	0	edema 3:4 cm
2 days	10	
3 "	180	
4 "	1600	
5 "		Edema over abdomen and chest
6 "		
7 "	1000	Edema smaller, hard, limited
8 "	0	
9 "	20	Only more infiltrate at the place of injection
10 "	Dead	

Dissection report: Purulent hemorrhagic infiltrate at place of injection, otherwise typical anthrax report.

We cannot be sure what causes this atypical infection course. We cannot say whether the individual resistance of the animal was abnormally high or whether -- which seems to be more probable here -- the virulence of the culture used in the infection was accidentally reduced. At any rate, the animal was half immune before it finally died as a result of the infection and this immunity could be recognized very nicely with the help of the positioning of the anthrax rods in the organs. In preparations stained according to Gram-Weigert we found very few anthrax rods in the liver; these rods were found exclusively in the Kupffer star cells. The cells themselves were swollen up and revealed indistinctly stained nucleii; they were quite visibly just as damaged as the bone marrow histiocytes in case of bone marrow infection. The anthrax bacilli were partly also damaged, which could be very easily recognized from the paler color, as well as the slimmer form or shape which no longer entirely corresponded to the picture of the animal

bacilli. At any rate, we were still able to find a relatively large number of well preserved anthrax bacilli. In addition to the previously mentioned highly desirable confirmation of the diagnosis of bone marrow puncture in other sections of the reticulo-endothelial system, we might mention in connection with this experiment -- which unfortunately can no longer be reproduced with certainty -- that an infection can die of anthrax infection without containing a major quantity of bacilli in the blood. This finding supports the view which we expressed in the beginning, to the effect that the damage to the reticulo-endothelium is the decisive factor in the infection and that the pathogenic effect of the anthrax bacilli emerges, as far as the vital parenchyma cells are concerned, only through the reticulo-endothelium. Since the bacilli were found exclusively in the cells of the reticulo-endothelial system, it had to be assumed that the immediate damage due to the infection likewise had to be confined to the reticulo-endothelium.

We were able to test this finding by means of the blockage experiment; in these experiments the selective damage to the reticulo-endothelial system weakened the [illegible]. We conducted three such experiments and we shall discuss them here.

Experiments 9 and 10

The highly immune animals, which had already successfully gone through several infections, were each injected 0.05 ccm of Bouillon culture, intravenously; they were able [illegible] this small amount without any disease phenomena. On the 14th, 15th, and 16th days after infection, each of the animals was given 10 ccm of [illegible], intravenously; on the 17th day, we performed a follow-up infection by administering 0.05 ccm of Bouillon culture, intravenously.

The animals revealed the following infection [sic; infection] course:

	Animal 1	Animal 2		Animal 1	Animal 2
30 Min.	60	[illegible]	2 days	Contaminated	300
1 hr. 30 min.	0	"	3 "	5	0
5 "	0	"	4 "	0	10
8 "	3	"	5 "	0	Dead
24 "	283	"		Lives	
36 "	500				

Rabbit 1 continues to live permanently; animal 2, on the other hand, died on the 5th day after infection. The finding for animal 2 was rather peculiar. From one leopold of [illegible] blood we obtained three anthrax colonies; on the other hand, cultures from the liver and spleen revealed grass-like growth. From this result we [illegible] conclude that the histological finding in animal 2 would [illegible] same as in the animal used for experiment 8. Principally we [illegible] to the effect of the blockage that this blockage or blockade [illegible] to in animal 1 whereas it had a more [illegible] effect in [illegible] of the infection with a quantity of bacilli which, 2 weeks [illegible], it had tolerated without any trouble at all.

Passive Immunity

The investigation of [illegible] passive anthrax immunity had to be confined to a few rabbits -- against such large infected quantities as we had [illegible] -- even the high-quality serum was found to remain ineffective, [illegible] administered several times. At any rate, we were able to establish with the help of intravenous infection attempts on passively immunized rabbits that, under the influence of immune serum, the bacilli, even animal bacilli, are retained longer in the reticulo-endothelia than in normal animals, and the protection derived from the immune serum with respect to similar infection doses can probably be traced to the fact that the anthrax bacilli, deprived of their attack substances, are held in the reticulo-endothelia and are finally killed.

Experiment 11

We take a rabbit and administer 5 ccm of rabbit immune serum, intravenously; 24 hours after the injection, the rabbit is given -- simultaneously with a control -- 0.05 ccm of bouillon culture in 1 ccm of cooking salt solution, intravenously.

	Immune Animal	Normal Animal
5 Min.	85	72
15 Min.	3	9
2 hrs.	9	9
8 "	9	9
24 "	9	∞
48 "	9	Dead
3 days	9	
4 "	9	
5 "	38	
6 "		
7 "	Dead	

By means of injection of immune serum we can prolong the life of the animal by five days, compared to that of the control animal. The viruses, which disappear into the reticulo-endothelium already after two hours, then remain there until the 5th day of infection. They occur in the blood of the animal in larger quantities only on the 6th day; after all of the protective forces, both the artificially introduced antiaggressive serum substances and the normal bactericidal capacity of the rabbit organism have been overcome, the animal is then killed by these viruses within 24 hours. We get the same picture when we infect the animals with animal bacilli.

Experiment 12

We centrifuge 2 ccm of rabbit plasma; the deposit is washed and is injected in equal portions into 4 animals, protected passively by 2 ccm of rabbit immune serum and into one control animal.

- - -

	[illegible]	[illegible]
[illegible] min	[illegible]	[illegible]
[illegible] "	[illegible]	69
1 [illegible]rs	[illegible]	[illegible]
[illegible] "	[illegible]	[illegible]
[illegible] "	-	6
[illegible] "	[illegible]	Dead, anthrax sepsis
	Dies of anthrax sepsis [illegible]	

In [illegible] immunity of the rabbits can be [illegible] reticulo-endothelial apparatus. The [illegible] of the [illegible] cells to this immunity is brought about not only [illegible] the antiagg[illegible]; we can also tell this [illegible] the fact that [illegible] the blood of the [illegible] animal [illegible] the [illegible] animal [illegible] [illegible] the [illegible] it protects [illegible] -- [illegible] -- condi[illegible] of [illegible] pointed out by [illegible].

[illegible]

[illegible] anthrax infection was explained [illegible] of these animals have the [illegible] of [illegible] the anthrax bacilli [illegible] cells of the [illegible] animal [illegible]. In the [illegible] not agree with the [illegible] of the problem [illegible] of bacilli. [illegible] these [illegible] immunity [illegible] the histiocytary [illegible] animal [illegible] considered as the [illegible] probable that [illegible] infection [illegible] a rabbit [illegible] the [illegible] proved that [illegible] destruction of saprophy[illegible] anthrax bacillus is a saprophy[illegible] naturally resistant chicken organism is concerned.

If we investigate the [illegible] infection in the chi[illegible] [illegible] can find that the course of [illegible] in the rabbit is very similar.

Experiment 13

We inject 0.2 ccm of anthrax bouillon into the wing vein of a hen. We take blood samples partly from the wing vein and partly from the skin veins in the neck.

5 Min.	52	9 hrs.	0 Sick, does not eat, feathers shaggy, diarrhea
15 "	0		
1 hrs.	0	24 "	0
3 "		48 "	0 healthy
6 "	0	Lives	

The anthrax bacilli disappear extremely quickly from the blood of the hen likewise but they can be found for a longer period of time in the parenchymatose organs. We were still able to establish a rather abundant quantity of anthrax viruses, by means of culture, in bone marrow removed one hour after the infection. It is rather questionable whether the temporary sickness of the hen was caused by anthrax infection. It is more probable that the frequent punctures -- which were very difficult in the case of the hen because the veins of the hen were very thin and would be easily torn, certainly had some effect on the well being of the animal.

A more detailed analysis of the defense processes resulting from bone marrow puncture is much easier in the hen than in the immune rabbit -- provided we do not narcotize the animals used for this experiment. In that case they can also tolerate large quantities of highly virulent anthrax while narcotized animals may die as a result of the infection, under certain circumstances.

Experiment 14

We rinse a 16-hour diagonal agar culture in 5 ccm of cookin salt solution; we inject 0.1 ccm of this substance into the bone marrow of the femur of a hen.

We take samples after 4 hours; culture positive.

Smear staining according to Giemsa.

We find individual histiocyterary cells which in most instances have absorbed very pale-dyed anthrax bacilli, and which usually reveal a large quantity of rods. The cells likewise are visibly damaged; they reveal a hyaline plasma; the nucleus is very often unevenly stained and always reveals an ameboid enlargement. Many anthrax cells are quite visibly in the last stages of dissolution; they are all entirely without capsules and they sometimes crumble in the form of granules. They are usually found in larger vacuoles which assume a pale-pink color following Giemsa staining; the extremely sparse free bacilli chains are still better preserved, throughout, than the phagocytized bacilli. They stain much more intensively; around the chains we usually find a free, unstained area which is very

similar to a capsule. In the methylene-blue preparations we find a large amount of metachromatically stainable substance which is located between the cells, in amorphous piles. The intracellular bacilli can no longer be illustrated with this staining but the extracellular can be illustrated.

We cannot find phagocytosis in other bone marrow cells.

These findings show us that the anthrax bacilli are absorbed by the histiocytes of the chicken just as much as they are absorbed by those of the immune rabbit. We think it is very probable that the killing takes place only in these cells because the chains, which are still located outside the cells, were definitely better preserved than those that had already been phagocytized. We were not sure as to the capsule formation of the anthrax bacillus in the organism of the chicken. At any rate, the unstained area around the free chains, along with the metachromatic substance which can be illustrated in the methylene-blue preparations -- a substance, by the way, which one usually ties in with capsule formation -- seems to indicate that we have a kind of animalization of the bacillus also in the organism of the chicken and that this process takes place in a fashion differing only slightly from the process to be found in the organism of receptive animals.

It was rather easy to perform the bone marrow infection in the hen; but judging the blockage [blockade] attempts [experiments] was very difficult. In streptococci and pneumococci we were able to determine the success of the blockade only by means of constant blood examinations in terms of culture growth. The same conditions would appear to obtain in the case of the blocked hen -- the only thing here is that this kind of constant control over the course of the infection was impossible. We said earlier that the veins tear very easily; this, plus the fact that veins, used for the India ink injections, develop thrombosis, made it impossible to keep up with the course of the infection. We had to be satisfied with being able to inject the hens intravenously with the required volume of India ink and to make a follow up infection in the same way. After this procedure, all of the veins that could be reached were already so altered that we could not possibly think of taking any blood samples.

Experiment 15

We take three hens and we inject them intravenously on 5 days with 50 ccm of 5% India ink, each, and after 24 hours we make a follow up infection, intravenously, with 0.2 ccm, each, of Bouillon culture. Here is the result of this experiment:

We had no success in two of these experiments, inasmuch as the hens continued to live. The third hen died on the fourth day after the infection with a positive culture report in the blood and in the organs; it is therefore quite obvious for us to assume that the two animals, who did not die of the infection, probably behaved in a fashion very similar to that of rabbit 1 in experiment 9.

In other words, we can say that, in a naturally resistant hen, likewise, blockage is followed by a weakening of the resistance -- especially since Bardach was already able to prove that the resistance of the dog can be broken through the intravenous injection of powered carbon. These experimental results are explained by Bardach who says that the majority of the phagocytes is so heavily taxed by the carbon particles that they can no longer absorb the anthrax bacilli and that, consequently, we have an extracellular multiplication of the injected bacilli. According to the experimental results, obtained by the author and Adler on pneumococci and according to the previously mentioned findings involving anthrax-immune but blocked rabbits, we must correct this opinion in one point. The reticulo-endothelial system does still completely absorb the bacilli but it has lost its capability for holding them in place and killing them. In blocked animals likewise we have a primary settlement and multiplication of the bacilli exclusively in the cells of the reticulo-endothelial system.

Experimental Results

We were able to trace the immunity of the rabbit and the natural resistance of the chicken against anthrax to the specific function of the histiocytary cell apparatus. This is an expansion on the findings by the author and Adler and Bass who proved the same for the cocci group, to the second group of sepsis viruses, that is, the aggressin forming agents. We furthermore proved that the immunity of the rabbit and the natural resistance of the chicken should be considered as basically identical forms of reaction; we believe that this is the last and most telling argument for the initially stated concept of immunity as a specific allergic alteration of the reticulo-endothelial apparatus. One might also state this view in the following way: the reticulo-endothelia are placed in a functional state, as a result of immunization, and this state is identical or at least highly similar to the state of the reticulo-endothelium of a naturally resistant animal.

Along with the works of Pfeiffer and Marx, Bieling and Isaac, as well as Neufeld and Meyer, who proved that the reticulo-endothelial system also produces the immune bodies, and along with the works of Wyssokowitsch and Rosenthal, who were able to prove that saprophytes introduced into the blood can be destroyed by the cells of this system, we can therefore recognize the overwhelming role of this cell system in the struggle of the organism against bacterial infection. Of course, this system does play a double role, in the sense that it can be considered as the seat of immunity, respectively, resistance, but that it also represents a refuge for the viruses in the animal organism and, under certain circumstances, allows them to multiply within the cells. The mechanism of infection and immunity is qualitatively identical; the only difference here is that in case of immunity it is the organism which is stronger whereas in case of infection it is the parasite which is stronger. The struggle is decided in the cells of the reticulo-endothelial system. We might very well imagine that we have a kind of rather tenuous balance here between the force of attack of the infection

virus and the defensive force of the organism; this balance or equilibrium might be preserved for a shorter or longer time; it would lead neither to the killing of the infection virus nor to a decisive damage to the infected organism; in the end, depending upon the environmental influences acting upon the organism, it leads to the predominance of the defensive forces if the organism is strengthened, or to a paralysis of the reticulo-endothelium, in case the resistance is reduced, with all of the consequences as far as the infected organism is concerned.

The blockade, which is followed by a weakening of the resistance, can be considered somewhat as a model for this kind of harmful environmental influence.

We can find a state of tenuous balance -- under certain circumstances featuring a temporary local predominance of the attacking force of the bacillus -- almost regularly in the anthrax infection of immune rabbits. Subcutaneous infection, after all, is frequently followed by a local multiplication of the viruses injected, accompanied by the formation of edema,, etc. The basic difference between this kind of infection in the immune animal and the infection process in the normal animal, which at first appears to be quite similar, can be found in the fact that, in the immune animal, there is no damage to the overall organism through the infection, whereas we can prove damage to the reticulo-endothelial system of the normal animal, as we said in the beginning, already 16 hours after the infection, in other words, even before the formation of the edema. This kind of early damage to the defensive apparatus -- which works against the infection both directly through phagocytosis and indirectly through its secretions, the immune bodies -- of course, cannot be immaterial in the fight against the local infection. If we now take a look at the reactions, which follow local anthrax infection, then we find that these reactions appear to be the same in the normal and in the immune animal and they prove a predominance of the attacking force of the bacillus over the local defensive functions. The decisive factor in the course of the infection is the damage to the total organism in the non-immunized animal while the immune animal is not damaged by the infection which progresses locally to a limited extent and thus gains time to mobilize its mobile defensive forces against the local process and to keep bringing up new reserves, whenever its defensive forces are rendered ineffective by the aggressins of the bacillus, while the damaged organism of the normal animal is paralyzed so early that such a reaction to the local process cannot take place and that the animal, in the end, succumbs to the infection, which now becomes septic, as was described by Bail and Weil and Suzuki.

The value of an existing immunity as far as the healing of a local infection is concerned will be confined to the extent to which a general impairment of the organism can be prevented. Once a locally infectious focus has been formed, we can say with certainty that the infection viruses have overcome the immunity of the infection site and have therefore moved beyond the range of this immunity altogether. In exsudates -- we use this term here in the broadest sense -- the immune forces no longer act directly upon the infection virus, as was pointed out in our discussion of pneumo-

coccus and streptococcus immunity. The healing heaing of a locally infectious affection now depends indirectly upon the immunity state. If the immunity is very high (pneumococci immunity) then the infection viruses are killed without infection; this happens for instance in the subcutaneous connective tissue or in the case of a rapidly starting and just as rapidly disappearing serous exsudation, such as in the pleural cavity. On the other hand, if the immunity is not that high, if it is not high enough to lead to the immediate killing of the parasites, as in the case of anthrax, then the viruses can move beyond the range of the immune forces in the developing exsudate; their killing is then left to the normal bactericydal forces of the organism which are possibly supported in this by the serum antibodies.

Summary

If ~~we inject~~ rabbits with virulent anthrax bacilli intravenously, these bacilli are absorbed by the cells of the reticulo-endothelial system. In the normal animal the bacilli multiply in these cells and penetrate from them, secondarily, into the blood. The cells of the reticulo-endothelial system are most severely damaged by this infection. If the animal is immune, the bacilli die in the cells. The conditions prevailing in the chicken are similar to those in the immune rabbit.

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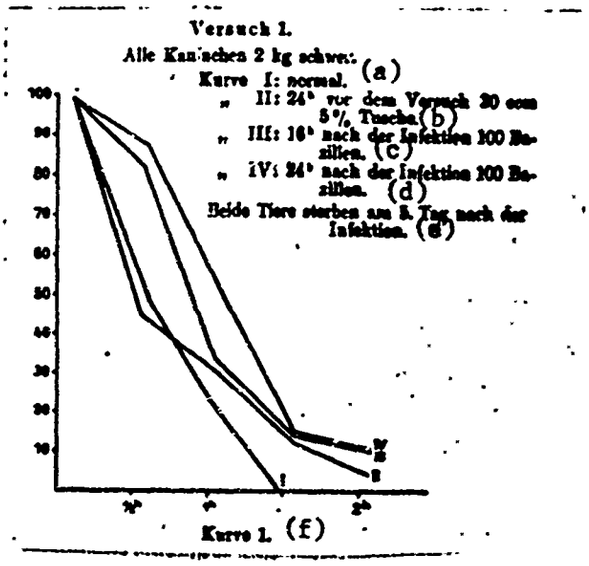
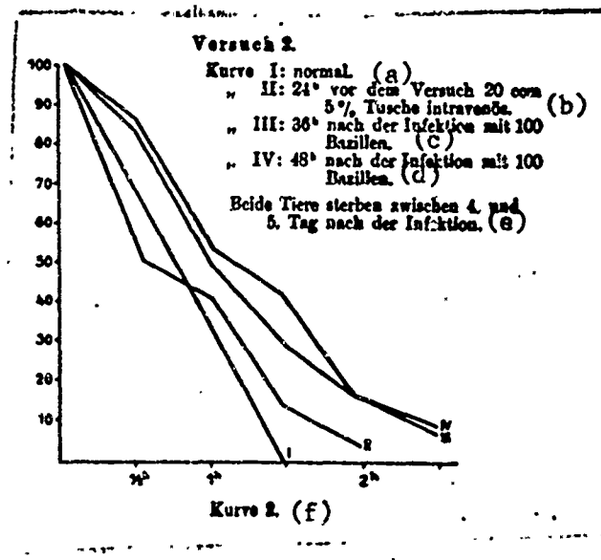
FIGURE APPENDIX

Experiment 1. All Rabbits Weigh 2 kg.



Legend: a -- Curve I: Normal; b -- Curve II: 24 hours before experiment, 20 ccm 5 % India ink; c -- Curve III: 16 hours after infection, 100 bacilli; d -- Curve IV: 24 hours after infection, 100 bacilli; e -- both animals died on 5th day following infection; f -- Curve 1.

Experiment 2.



Legend: a -- Curve I: Normal; b -- Curve II: 24 hours after experiment, 20 ccm 5 % India ink, intravenous; c -- Curve III: 36 hours after infection with 100 bacilli; d -- Curve IV: 48 hours after infection with 100 bacilli; e -- Both animals died between 4th and 4th days after infection; f -- Curve II.